Myosin V takes multiple 36-nm steps along an actin filament by coordinating its two motor domains. The properties of myosin V have been addressed by means of several assays. In particular, optical tweezers techniques and FIONA have contributed widely to enlightening the motion mechanism. However, these two assays cannot be applied when studying the mechanism for coordinated force generation by a single motor domain during motion. For myosin V, optical tweezers assays are restricted to studying the mechanical properties of both motor domains when an optically-trapped bead is attached to the Myosin V tail. On the other hand, although FIONA is capable of observing the motion of a single motor domain during gait motion, it is not capable of revealing the mechanical properties because these studies do not apply external force.
Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V based on the previous report by Block's lab. The DNA linker is used to connect a bead to one of the two motor domains. In this experimental setup, external force by the optical tweezers is applied to a single motor domain directly via the DNA linker during gait motion. By using this measurement system, we succeeded to observe single head behavior while force is applied to it.

2902-Pos
Reconstituting a Native Actin Track for Myosin V Transport
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The budding yeast *S. cerevisiae* is an excellent model system for the study of cargo transport by myosin, given the relative importance of actin cables versus microtubules in this cell. Despite this, no *in vitro* studies have tried to mimic the actin-tropomyosin cables along which the class V myosin Myo2p transports secretory vesicles, vacuoles, mitochondria, and other organelles to the growing bud. We find that Myo2p is non-processive *in vitro*,in agreement with previous results [1-2]. These experiments were, however, performed using chicken skeletal actin, which is only 87% identical to yeast actin. Accordingly, we are investigating if Myo2p behavior changes when the *in vitro* conditions more closely match those found in the yeast cell. Actin cables will be reconstituted *in vitro* from yeast actin, yeast tropomyosin, and the actin bundling protein fimbrin. The effects of yeast versus skeletal actin, of bundled actin versus single filaments, and of the presence of each of the two different tropomysin isoforms will be tested. Myo2p motility, processivity, and actin binding affinity will be assessed with these different tracks. The effect of varying ionic conditions, nucleotide concentration, and viscosity will also be tested, to determine if Myo2p behavior changes as conditions more closely match those of the intracellular milieu.
1. Reck-Peterson et al., *JCB* 153 (2001).
2. Dunn et al., *JCB* 178 (2007).

2903-Pos
Liposomes as Model Cargo for Myosin Va
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Myosin Va (myoVa) is a processive, actin-based motor involved in the transport of membrane bound secretory vesicles and organelles. How multiple motors attached to such cargo generate productive forward motion is unclear. To address this, we have coupled expressed myoVa-HMM with a C-terminal biotin tag to extruded fluorescent, neutravidin-coated liposomes as an in vitro model for intracellular cargo. This model system allows control over liposome size, fluidity, and surface density of attached motors. When observed in TIRF on actin tracks at room temperature, "rigid" 400nm DPPC liposomes with ~160 motors/liposome move at speeds equal to that of a single processive myoVa-HMM (510 ± 227nm/s), whereas, 200nm liposomes with the same surface density of motors move 30% slower (352 ± 121nm/s). In comparison, "softer" 200nm DMPC/cholesterol liposomes that have more fluid phase membranes are slower yet (229 ± 130nm/s). These velocity data suggest a complex relationship between the ensemble of attached motors and the liposome rigidity/fluidity and size. With more fluid membranes, motors may be mobile within the liposome membrane, compromising their contribution to forward motion and thus the slower velocities. In contrast, larger, more rigid liposomes may allow a fixed number of transporters to remain productively engaged. Interestingly, liposomes can be observed "cartwheeling" along actin tracks, suggesting that motors can exchange roles between being an active transporter and a passenger waiting its turn as the liposome effectively rolls down the actin track. By attaching Qdot-labeled myoVa-HMM, the exact spatial relationship between the motor and liposome cargo can be determined to help understand and model the complexities of this simplified in vitro representation of intracellular cargo transport.

2904-Pos
Myosin Va Cargo Transport on Actin Bundles
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Myosin Va (myoVa) walks processively while carrying cargo towards the plus end of actin filaments. In cells, parallel actin filament bundles (e.g. stress fibers and filopodia) present a directional challenge to myoVa cargo transport. Therefore, we formed unipolar (fascin) and mixed polarity (alpha-actinin) actin bundles as tracks for expressed myoVa-HMM with a C-terminal biotin tag. In this assay, a single streptavidin-Qdot served as cargo for one or many (~ 5) myoVa motors. Qdots transported by one or many myoVa molecules traveled in the same direction on unipolar bundles, while moving in either direction on mixed polarity bundles. Qdot speeds were the same regardless of bundle type or number of motors (400nm/s), and similar to that for one or many motors on a single actin filament (Nelson et al., 2009). However, run lengths for single motors were 2-3 times longer on bundles than previously observed on single actin filaments. This suggests that on parallel tracks the leading head has a greater number of actins within its reach, thus decreasing the probability of run termination. Interestingly, on mixed polarity bundles, we observed individual Qdots changing directions in the middle of a run, the frequency of which increases in the multiple motor case. It was not surprising that a Qdot with a single motor can switch directions on a mixed polarity bundle, given myoVa's inherent flexibility that allows it to turn up to 150o at actin filament intersections (Ali et al., 2007). These data also suggest that one or many myoVa molecules bound to a single cargo have the ability to jump tracks to neighboring actin filaments. With Qdot-labeling of the individual heads, high spatial resolution studies will confirm this on mixed polarity bundles, and determine whether the motors also wander on unipolar bundles.

2905-Pos
Flexibility of Stepping Manner of Myosin V and X Processive Movement on 2D Actin Structures
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In a TIRF *in vitro* motility assay, we investigated the processivity and stepping characteristics of myosin V HMM and myosin X HMM with a leucine zipper on single actin filaments and actin bundles. Actin was polymerized and cross-linked on a charged lipid monolayer in Teflon wells in order to create regular 2D or 3D structures. Two cross-linking proteins were used: alpha-actinin which produces non-polarized bundles with 40 nm filament spacing and fimbrin which produces polarized actin bundles with 14 nm filament spacing. We were determined the velocities and the run length for processive movement on those 2-D actin bundles by using modified particle tracking softwear. Myosin V moved processively on all types of in vitro actin structures. Myosin X moved well on polarized fimbrin cross-linked bundles but movement was impaired or non-existent on non-polarized alpha-actinin bundles. Furthermore, we have measured the stepping manner of myosin V and X by using FIONA analysis, which allow us to measured nano-meter precision. Myosin V steps along single actin filaments, while myosin X steps over several actin filaments on the 2-D actin filaments. We hypothesize that forward runs of myosin X on alpha-actinin cross-linked bundles are inhibited because myosin X "sidesteps" to a parallel oppositely polarized filament and the run stalls. The presence of a SAH domain in the lever arm of myosin X could increase the working stroke or flexibility of the lever arm and allow it more easily sidestep the larger alpha-actinin filament spacing.

2906-Pos
Mechanical and Kinetic Properties of a Myosin 5-SAH Chimera
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We have determined the kinetic and motile properties of a myosin 5a HMM construct in which four calmodulin-binding IQ motifs are replaced by the putative single alpha helical domain (SAH) of similar length from Dictyostelium myosin, MyoM. Electron microscopy of this chimera showed that the SAH domain was straight and 17 nm long as predicted, restoring the truncated lever to the length of wild type (Myo5-6IQ). The powerstroke (21.5 nm) measured in

the optical trap was slightly less than that for Myo5-6IQ (25 nm) but much greater than for Myo5-2IQ (10 nm). Myo5-2IQ-SAH moves processively along actin at physiological ATP concentrations with similar stride length to Myo5-6IQ in TIRF microscopy assays, and the average run length was also similar. Stopped-flow fluorescence experiments indicated that unlike WT Myo5-6IQ the rear head did not mechanically gate the rate of ADP release from the lead head of the chimera and the rate of ADP dissociation was the same from both heads. These data show that the SAH domain can form part of a functional lever in myosins although its bending stiffness might be lower. We conclude that SAH domains can act as mechanically-competent structural extensions in physiological conditions and that gated dissociation of ADP from the lead head of myosin 5 is not required for processive movement.
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Electron & Proton Transfer

2907-Pos

Functional and Protein Processing Insights from a Truncation Mutation in Subunit III of Cytochrome *c* Oxidase from *Rhodobacter sphaeroides*

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A human mitochondrial DNA mutation in the Cytochrome c Oxidase (COX) subunit III (SIII) gene causes a truncation after its three n-terminal transmembrane helices and results phenotypically in severe lactic acidosis episodes. We created the equivalent mutation at position I115 in SIII of *R. sphaeroides* COX (I115stop). Truncated SIII enzyme was expressed and purified, and SDS-PAGE showed an absence of full length SIII and a doublet band of lower molecular weight which was immunoreactive to a SIII site-specific antibody. MALDI-TOF determined these peptide masses to be 12919 m/z and 11462 m/z, results consistent with a I115 truncation in SIII (12915 m/z) and subsequent proteolytic processing after F101 (11461 m/z). Wildtype COX subunit II is known to undergo protease processing *in vivo*, yielding different forms of the subunit (IIA, IIC). SDS-PAGE and MALDI-TOF showed higher levels of the less-processed IIC form in I115stop mutant preparations as compared to wildtype, which had higher levels of the IIA form. Functional assays show the I115stop mutant has a maximal electron transfer activity that is approximately 30% of wildtype (480 ± 90 e-/s*mol versus 1670 ± 180 e-/s*mol) and exhibits suicide inactivation similar to a form of the enzyme lacking SIII altogether (I/II_{OX}). The first three helices of SIII putatively contain conserved lipid binding sites, so the electron assays were then conducted in the presence of exogenous lipids. The I115stop mutant exhibited a greater stimulation of activity due to lipid than I/II_{OX} (23% versus 5%). Additionally, protection from suicide inactivation by lipid was 2.4 fold greater in the I115stop mutant than I/II_{OX}. Taken together, the results indicate that the truncation mutation alters native subunit II c-terminal processing, and they support the hypothesis that SIII is involved in functional lipid binding.

2908-Pos

Detection of a Proton-Dependent Electron Transfer from Cu_A to Heme a of Cytochrome C Oxidase Mutant S44e Using Ruthenium Photoexcitation

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Crystal structures, sequences, and homology models of mammalian, yeast, wheat, and *Thermus thermophilus* cytochrome c oxidases show a conserved glycine hydrogen bonded to a heme a histidine ligand, while the bacterial oxidases from *Pd* and *Rs* offer the hydroxyl group from a serine (S44) for hydrogen-bonding to the H102. In order to study the effects on electron transfer due to mutation of this position to a glutamate, a photoactivatable Ru probe was attached to cytochrome c, the natural redox partner of oxidase. A laser flash of less than 0.5 us reduced cytochrome c and allowed the measurement of individual steps of electron transfer from cytochrome c to Cu_A to heme a. The mutant exhibited two phases in the rate of electron transfer from Cu_A to heme a. Both phases had amplitudes and rates that were highly dependent upon pH indicating a protonation-deprotonation event of the glutamic acid residue. In combination with previous data obtained from other S44 mutants, including the S44D mutant, these results indicate that the heme a redox potential can be dramatically altered by a nearby carboxyl and its protonation leads to a proton-coupled electron transfer process. This work was supported by grants GM26916, GM20488 and NCRR COBRE 1 P20 RR15569.

2909-Pos

Exciton Interactions Between Hemes b_n and b_p in the Cytochrome b_6f Complex

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Circular dichroism spectra have been previously utilized (1) to analyze heme-heme interactions of the two *b*-hemes in the mitochondrial bc_1 complex that were predicted to bridge the 'B' and 'D' trans-membrane helices on the *n*- and *p*-sides of the cytochrome *bc* complexes (2). It was of interest in the context of the 3.0 Å structure of the b_6f complex (3-6) and its unique bound chromophoric prosthetic groups, Chl *a* that is 12 Å from heme b_p, and heme c_n that shares electrons with heme b_n, to analyze CD spectra of the heme Soret bands in crystallization-quality b_6f complex. Sources of the cytochrome b_6f complex were the cyanobacteria, *M. laminosus* and *Nostoc* PCC sp. 7120, and spinach thylakoids. In the crystal structures, the oxidized *b* hemes are separated by 20-21Å center-center and 7-8Å, edge to edge, and rotated relative to each other by approximately 55° about an axis almost normal to the membrane plane. A bi-lobed dithionite minus ascorbate-reduced CD difference spectrum, qualitatively similar to that seen in the mitochondrial bc_1 complex, was obtained from all three sources. Positive and negative bands on the blue and red sides of a 431 nm node, the peak of the absorbance difference spectrum, are diagnostic of excitonic heme-heme interactions. There is no significant contribution to these difference spectra from the Chl *a*, heme c_n, or the heme of cytochrome *f*. *deceased; 1, Palmer and Degli-Esposti, 1994; 2, Widger *et al*. 1984; 3, Kurisu *et al*. 2003; 4, Stroebel *et al*. 2003; 5, Yamashita *et al*. 2007; 6, Baniulis *et al*. 2009.

2910-Pos

Do Tyrosine Phenolic Groups Contribute to the Alkaline Transition in the Redox Potential of Cytochrome F?

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Cytochrome f, a c-type cytochrome involved in the photosynthetic electron transport chain, has a significantly higher redox potential than most other c-type cytochromes, ranging +370 to +380 mV. Like cytochrome c, cytochrome f also exhibits an alkaline transition in which the redox potential becomes pH dependent at high pH. In the case of cytochrome c, this has been attributed to replacement of the methionine sulfur serving as the sixth iron ligand by a deprotonated amino group. This cannot be the cause for the alkaline transition in cytochrome f as there is no methionine ligand to the iron, the sixth position being occupied by the N-terminal amino group. Three tyrosine phenolic groups (Y1, Y9, and Y160) are found in close proximity to the heme in the cytochrome f structure. To explore the possibility that the ionization of one or more of these tyrosines might be responsible for the alkaline transition, we have performed site directed mutagenesis, replacing each with a phenylalanine residue which lacks an ionizible group. Each of these mutants was found to have a redox potential of 375-380 mV at pH 7.0 which became pH dependent above pH 9.0 (apparent pKa 9.3). It thus seems unlikely that any of these tyrosine residues contributes to the alkaline transition of the redox potentials in cytochrome f. Redox properties of Y160L and R156L mutants will also be described.

2911-Pos

Characterization of the Secondary Quinone (Q_B) Binding Pocket in Photosynthetic Reaction Centers Using Pulsed EPR Spectroscopy

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3-pulse ESEEM and HYSCORE pulse sequences have been used to analyze the secondary electron acceptor semiquinone anion radical (Q_B^-). Photosynthetic reaction centers from *Rhodobacter sphaeroides* have identical ubiquinone molecules functioning as primary and secondary electron acceptors. The primary quinone radical (Q_A^-) has been extensively studied, and hydrogen bonds have been characterized at both carbonyls. The structure around Q_B^- has received less attention. The O_4 carbonyl has been suggested to be hydrogen bonded to the N_δ from a histidine at residue L190. The O_1 carbonyl also possesses a hydrogen bond that is weaker than that at O_4. OH from serine at L223 is important in the hydrogen bond structure at this carbonyl. However, contributions from surrounding peptide nitrogens are suggested by x-ray structures but have not yet been investigated by EPR methods. Pulsed EPR studies of the Q_B^- radical confirm one strongly coupled nitrogen with NQI frequencies consistent with a histidine N_δ. 3-pulse ESEEM and HYSCORE spectra also contain peaks from a second nitrogen nucleus. *A priori* knowledge of the origins of these peaks is less clear, but could include contributions from a backbone nitrogen. Additionally, NQI modulation from ^{14}N is sufficiently shallow to observe